Accepted Manuscript

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PII: S0924-2244(18)30933-6

DOI: https://doi.org/10.1016/j.tifs.2019.07.023

Reference: TIFS 2566

To appear in: *Trends in Food Science & Technology*

Received Date: 8 January 2019

Revised Date: 9 May 2019

Accepted Date: 19 July 2019

Please cite this article as: Panthi, R.R, Kelly, A.L, O'Callaghan, D.J, Sheehan, J.J, Measurement of syneretic properties of rennet-induced curds and impact of factors such as concentration of milk: a review, *Trends in Food Science & Technology*, https://doi.org/10.1016/j.tifs.2019.07.023.

Abstract

Background

The rate or extent of whey expulsion or syneresis from cheese curds during stirring in-vat determines curd moisture levels, which subsequently influences cheese moisture content. The outward migration of whey depends on curd contraction and on the structure of the pores permitting whey movement. Curd syneretic properties are one of the least understood areas of cheese science, particularly when milk of varying composition is used.

Scope and Approach

This review provides an insight into the mechanisms of curd formation and curd syneresis, and factors influencing syneretic properties in unconcentrated and concentrated milk and appraises syneresis measurement methods in terms of their relative strengths and weaknesses.

Key Findings and Conclusions

Direct measurement of moisture content of curds is recommended as a simple and reliable method for measurement of syneresis of industrial relevance and, although inline measurement for curd moisture prediction has been a significant development in the last decade, its application to commercial production is still limited. A review of previous studies found that experimental conditions and methodologies used to measure syneresis vary widely, making it difficult to compare data between studies. Overall, interactions between process variables employed determines whether syneresis is accentuated or inhibited, and this can be exploited by cheese producers to attain target curd moisture contents by varying process parameters,

particularly when milk is concentrated prior to cheese-making. Furthermore, further studies should be focused on endogenous syneresis and casein network rearrangement to clearly elucidate this mechanism and its influence on macrosyneresis under dynamic conditions.

Measurement of syneretic properties of rennet-induced curds and impact of factors such as concentration of milk: a review

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1. Introduction

In cheese-making, the rennet-induced coagulum is cut into small cubes of curd (5 to 15 mm) and subsequently stirred in-vat to release a large proportion of the occluded whey (syneresis). Expulsion of whey from the curds during stirring influences curd moisture content prior to drainage, and thereby the final moisture content in cheese (Everard et al., 2008; Giroux, Bouchard, & Britten, 2014). For instance, in making hard-type cheese, a lower level of curd moisture content is targeted than in making soft-type cheese, i.e., higher moisture varieties.

Whey expulsion from cheese curds during in-vat stirring is a complex phenomenon which is influenced by multiple conditions prevailing in the cheese vat, e.g., milk composition, curd firmness, curd particle size, stirring speed, stirring time, temperature, acidity, enzyme concentration, $CaCl_2$ addition, and milk pH (Kaytanli, Erdem, & Tamer, 1994; Walstra, Van Dijk, & Geurts, 1985). The conditions which increase collisions between curd particles and their deformation in a cheese vat directly affect the rate of whey expulsion and hence moisture content at a given time (Jimenez-Marquez, Thibault, & Lacroix, 2005). Whey release from rennet-coagulated curds is due to their contraction (Ozilgen & Kauten, 1994; Renault, Gastaldi, Lagaude, Cuq, & delaFuente, 1997), which is related to the microstructural properties of curds, e.g., porosity and permeability (Walstra et al., 1985), as well as biochemical makeup, e.g., surface properties of casein micelle (Pearse & Mackinlay, 1989) and curd rheology (Castillo, Lucey, Wang, & Payne, 2006).

Where cows are fed controlled diets, variation in milk composition is minimal (Heck, van Valenberg, Dijkstra, & van Hooijdonk, 2009). However, where natural variation in milk composition occurs due to breed, herd, feed or seasonality, consistency in final cheese quality, e.g., cheese moisture content, is hard to achieve (Amenu & Deeth, 2007). Generally, process

steps follow standard operating procedures (SOP), and variation in milk composition for cheese-making results in different coagulation and whey expulsion behaviours (Castillo et al., 2006; Mateo et al., 2009c), providing a significant challenge in following SOP protocols. The ability to predict or control the syneresis of curds, despite changes in milk composition, has gained attention recently (Jimenez-Marquez et al., 2005; Mateo et al., 2009b), with a view to allowing cheese producers to make decisions to achieve appropriate curd moisture content prior to drainage.

Membrane filtration technology allows concentration of milk components to higher levels, which can facilitate increased factory throughput (depending on the milk concentration factor) using existing resources (Kevany & Guinee, 2018; Ong, Dagastine, Kentish, & Gras, 2013a). Use of ultrafiltration systems for protein standardization by the cheese-industry prior to cheese-making will not be unusual in the future for increasing consistency and productivity (Guinee, O'Kennedy, & Kelly, 2006). Much research is now focused on studying cheese-making using milk with higher solids contents (Caron, Pouliot, & St-Gelais, 2001; Govindasamy-Lucey, Jaeggi, Martinelli, Johnson, & Lucey, 2011; Ong et al., 2013a). However, curd syneretic properties is one of the least understood areas in cheese science, particularly when milk of different composition is used or where milk is concentrated to higher protein levels. To enhance the understanding of the curd properties, this review provides an insight into the mechanism of rennet-induced curd syneresis, factors influencing syneresis properties in unconcentrated and concentrated milk, and summarises syneresis measurement methods in terms of their relative strengths and weaknesses.

2. Gel formation

After the addition of rennet to cheese-milk, the hydrophilic caseino-macropeptide moiety of κ-casein is removed, destabilizing casein micelles. The destabilized casein micelles are connected initially in the form of dimers and trimers. Subsequently, a three-dimensional protein network is developed through Ca^{++} bridges, entrapping fat, moisture and minerals (Choi, Horne, & Lucey, 2015; Dejmek & Walstra, 2004). A schematic presentation of changes that occur in casein micelles and the corresponding rheological properties observed in rheometry are shown in Figure 1.

When a renneted milk sample is monitored by rheometry, at the early stages it does not show any consistency in signal, which continues for the time taken by rennet to hydrolyse the casein micelles. The sample first exhibits some strain, with an apparent increase in storage modulus (G'), when hydrolysed casein micelles start forming aggregates through Ca^{++} bridges. Although various studies have defined rennet coagulation time (RCT) as the time when viscous modulus (G") is equal to elastic modulus (G') of a gel, the first consistent increase in G' can also be taken as the RCT. The storage modulus of the gel increases continuously due to increased networking of hydrolysed casein micelles in the 3-dimensional protein network. Mellema, Walstra, van Opheusden, and van Vliet (2002) proposed that movement of casein micelles or clusters by detaching at weak bonds at one junction and connecting to another junction eventually leads to increased G' values. Therefore, an increase in G' at a given time is due to increased strength of the bonds between micelles, which is due in part to the rearrangement of casein micelles in the network.

The firmness of gels for cheese-making is an important criterion. The gels should be capable of entrapping fat and resisting curd breakage, as well as being sufficiently flexible to expel entrapped moisture. The rate of increase in gel firmness ($\Delta G'/\Delta t$) is positively influenced by

increases in the level of various factors, such as levels of protein, calcium, rennet concentration, coagulation temperature, and can be negatively influenced by increasing pH. The influence of milk composition on rennet coagulation properties was discussed in recent papers (Horne & Lucey, 2017; Panthi, Jordan, Kelly, & Sheehan, 2017). In typical uncut casein gels, around 10% of the water is present in casein micelles and around 90% is entrapped in the protein network (Van Vliet & Walstra, 1994). After a gel reaches the appropriate firmness, the coagulum is cut to release the whey. The majority of water present in curds is expelled to attain desired final moisture level of curds prior to drainage

However, an investigation of the interactive effects of factors influencing rennet coagulation properties may provide a greater insight to cheese producers as to how to achieve suitable gel properties when milk of varying composition is used.

3 Physicochemical mechanisms of gel rearrangement and shrinkage

Understanding the physicochemical mechanisms of gelation and the rearrangement of the protein network is useful to monitor the dynamics of whey expulsion during cheesemaking (Lucey, 2002). However, since the casein network is highly unstable during the earlier stages of gel formation, it is hard to elucidate the mechanism of rearrangement clearly. It is accepted that rearrangement of the protein network exerts a force that may apply pressure to whey, resulting in so-called endogenous syneresis pressure (Van Dijk, 1982). As casein micelles are reactive on their entire surface, such rearrangement may initially be caused by those casein micelles that are not connected in the network. Moreover, in the later stages, the extensive breaking of the bond between *para*-casein micelles may be responsible for causing rearrangement (Mellemma et al., 2002). This rearrangement of the casein clusters in the three-dimensional network provides the primary force (~1 Pa) for curd shrinkage, in the absence of an externally applied force (Van den

Bijgraat, 1988). However, when the shrinkage is constrained, the network rearrangement produces an inhomogeneous structure, resulting in increased gel permeability, which favours whey migration within the gel, inducing microsyneresis (Van Dijk, 1982).

The endogenous syneresis pressure has been reported to increase with the gel permeability and whey expulsion kinetics, leading to curd shrinkage (Castillo et al., 2006; Van Dijk, 1982). This increases over time, reaches a maximum and finally decreases, according to the dynamics of *para*-casein bond relaxation in the network. Therefore, change in endogenous syneresis pressure can be linked with casein micelle mobility and the inter micellar distance, which increases at lower pH, higher temperature and increasing volume fraction (Van den Bijgraat, 1988).

Rearrangement of casein micelles in rennet-induced gels may be well described by correlation with tan δ values calculated from the ratio of loss modulus to storage modulus of a gel (Van Vliet, Van Dijk, Zoon, & Walstra, 1991). Theoretically, the permeability of a gel prepared from concentrated milk should increase due to the greater number of protein-protein bonds in a junction, which are likely to be involved in greater rearrangement. However, tan δ values measured after 40 min in gels prepared from 4 to 6% protein did not differ (Panthi, Kelly, Sheehan, Bulbul, Vollmer & McMahon, 2019), suggesting that protein rearragnement could occur in the early stages of gelation. When casein micelles are sufficiently fused, the timescale for such network rearrangement may become longer, decreasing the endogenous pressure (Van Vliet et al., 1991). Hence, it is proposed that endogenous syneresis, at a given time in a gel, could be a balance between casein micelle rearrangement and their permanent fusion.

However, cheese curds are poroelastic in nature, which means that the network becomes denser over time, as a result of structural changes in the protein matrix. Tellier, Mariette,

Guillement, and Marchal (1993) reported that the diameters of porous channels decreased from 100 nm to 50 nm in a rennet-induced gel during contraction. In contrast, van Dijk (1982) previously demonstrated that the permeability of curd increases over time when measuring the volume of whey on the top while injecting whey from the bottom of the curd column. The differences observed between studies could be due to the application of different methods, as injecting whey through a curd sample may cause some destruction in the protein network in the study of Van Dijk (1982). Nevertheless, it could be assumed that permeability of a curd during cheesemaking decreases due to curd shrinkage around its surface to a greater extent compared to the curd interior during cheesemaking. Hence, the dynamics of change in permeability and porosity of the curd particles are important physical properties which can restrict the movement of whey through the open channels (Castillo et al., 2006).

Tellier et al. (1993) did not observe whey expulsion in a gel formed in a narrow (10-mm diameter) glass tube. The possible explanation for this mechanism is adhesion of the gel to the wall of the glass tube, which restricts network contraction (Dejmek & Walstra, 2004). In line with the dynamics of curd contraction during syneresis, the rate of whey expulsion is higher at the beginning of stirring and becomes slower over time (Mateo et al., 2009c; Ozilgen & Kauten, 1994). Since cheese-making is mainly a dehydration process, the occluded whey in the protein network is ‘squeezed’ out by changing process parameters which favour flow of whey through the porous surface of the curd. Moreover, whey expulsion from a gel is probably limited based on the permeability at the surface of the gel but deformation applied on cheese curds during stirring probably mask such small contribution of endogenous syneresis for whey expulsion (Van den Bijgraat, 1988).

Gel/curd properties such as endogenous syneresis pressure, protein network rearrangement, and dynamics of permeability have been the focus of few studies (Castillo et al., 2006; Choi et al., 2015; Mellemma et al., 2002; van Dijk, 1982; Van den Bijgraat 1988); thus, there is a requirement for further research in this area under controlled experimental conditions to improve its understanding. Application of electron microscopy to visualize the dynamics of such changes have significantly contributed to the interpretation of rearrangement phenomena in recent years (Castillo et al., 2006; Mellemma et al., 2002; Panthi et al., 2019).

4. Curd microstructure

The structure of a rennet-induced gel during cheese making influences almost all processing steps down-stream during the cheese-making process and has been the focus of much research over the past decade (Castillo et al., 2006), particularly on how changes in the gel structure impact cheese-making. During cheese-making, achieving appropriate gel characteristics are important to entrap fat globules in the protein network and to facilitate contraction after cutting of the coagulum. Increasing coagulation temperature results in inhomogeneous and coarse structure (Castillo et al., 2006). Milk coagulated at lower temperatures (27°C) results in a gel with a finer protein network than milk coagulated at higher temperatures (36^{o}C) (Ong, Dagastine, Auty, Kentish, & Gras, 2011) (Figure 2 A, B). The latter also results in significantly higher fat losses to whey than the former during cheese-making, which is attributed in part to the formation of a coarse structure or to the increased flexibility of fat globules at the higher temperature, i.e., above the melting points of certain fatty acids (Ong et al., 2011). These phenomena could partly be explained by the enhanced rearrangement of casein micelles at higher coagulation temperature.

Hussain, Grandison, and Bell (2012) reported smaller pores in milk gels made from bovine or buffalo milk at 34°C compared to higher or lower set temperatures. Milk derived from different species (e.g., buffalo or goat) yields gels with different microstructure, owing to differences in milk composition (Hussain et al., 2012; Rovira, Lopez, Ferrandini, & Laencina, 2011), suggesting that the cheese-making parameters should differ for milk derived from different species. For example, under similar gelling conditions, gels prepared from bovine milk show higher porosity than gels prepared from buffalo milk (Hussain et al., 2012). As moisture is mainly entrapped in the porous structure of a gel (Van Vliet & Walstra, 1994), the presence of higher moisture levels in a gel results in a more porous structure and lower strength.

Calcium is added to cheese milk for promoting protein-protein linkages, which provides strength in the protein network. The formation and mobility of bonds between micelles is promoted when caseins are cross-linked with less insoluble Ca (Choi et al., 2015). Cheese curds containing increased Ca levels have been reported to shrink less (Geng, van den Berg, Bager, & Ipsen, 2011). In contrast Ong, Dagastine, Kentish, & Gras, (2013b) did not observe the apparent effect of increased levels of Ca^{++} on the gel structure, however, the marked effect of Ca was observed in cooked curds, with the formation of a dense protein network. The influence of calcium on milk pH could also play an indirect role in altering the curd microstructure.

Geng et al. (2011) reported that application large deformation on a curd could break the protein network resulting in the formation of unbranched denser curd structure, which probably retartd the curd shrinkage.

5. Factors influencing syneresis

Factors influencing syneresis can be categorized into intrinsic and extrinsic factors, where intrinsic refers to those factors that affect syneresis of cheese curds due to changes in milk

composition (e.g., fat level, protein level, minerals level) and extrinsic factors are those which are applied externally to the cheese curds (curd size, stirring speed, temperature, cooking, etc). Such factors influencing the syneresis properties of curds have been extensively reviewed previously (Dejmek and Waltra, 2004), and thus have only been discussed briefly in this review, and the influence of enzymes, starter culture, milk pre-treatments, and colloidal calcium phosphate have not been covered.

Various studies have reported that decreasing milk pH increases the amount of released whey (Grundelius, Lodaite, Ostergren, Paulsson, & Dejmek, 2000; Kaytanli et al., 1994; Piyasena & Chambers, 2003; Piyasena, Chambers, Reekie, Feirtag, & Tung, 2003), as a result of increased curd shrinkage (Lodaite, Ostergren, Paulsson, & Dejmek, 2000). Increasing coagulation temperature during cheesemaking increases the rate of whey expulsion (Castillo et al., 2006; Giroux et al., 2014). Inclusion of additional whey protein (Piyasena et al., 2003) or any water-binding materials, e.g., starch (Brown, McManus, and McMahon, 2012), or exopolysaccharide from bacteria (Costa et al., 2012), results in increased hydration and hence reduced whey expulsion. However, milk fat has a complex effect on curd moisture; it may reduce the curd moisture contents as the proportion of fat content in milk increases (Arango, Trujillo, & Castillo, 2015; Everard et al., 2011; Mateo et al., 2009c; Talens et al., 2010) or it may increase moisture levels of the non-fat substances by closing porous channels and thereby impeding moisture flow during syneresis (Mateo et al., 2009c). Increased levels of $CaCl_2$ in milk also retard curd shrinkage, due to the formation of a rigid protein-protein network (Geng et al., 2011), and hence may result in reduced syneresis.

Cutting and stirring of curds have a significant role in syneresis in cheese-making and final cheese moisture content. Trials from a commercial cheese processing plant showed that,

with increasing cutting speed (i.e., increased revolutions per min of the cheese knives) cheese moisture content decreased (Jimenez-Marquez et al., 2005), probably due to the formation of smaller curd particle sizes with increased curd surface area (Grundelius et al., 2000; Renault et al., 1997). Lodaite et al. (2000) reported a greater proportion of curd shrinkage in curd with a smaller thickness (3 mm) compared to curd slabs with greater thickness when keeping all other factors constant, including surface area. It was not clear which force played a role in contraction of the thinner curd slab to a greater extent; however, it is likely that surface wetting using permeate on the curd slabs of different thickness could exert a different level of pressure. Generally, curd particles are smaller in size (1.73 to 2.72 mm^2) before drainage (Iezzi et al., 2012) for the manufacture of hard compared to soft cheese varieties. However, practices of cutting curds at different sizes for different cheese-varieties are not common, which is usually achieved by changing cutting knife speed (rpm) (Everard et al., 2008; Johnston, Dunlop, & Lawson, 1991) which can also induce more frequent collisions, resulting in large deformation forces, favouring more whey expulsion. In-vat curd moisture content before curd drainage can be manipulated by changing the factors influencing the syneresis (Arango et al., 2015; Calvo & Balcones, 2000; Jimenez-Marquez et al., 2005).

Vat configuration also influences the cut sizes. Ost vats (vats where the cutting knife is located on a horizontal shaft in the middle of cheese vat) have been shown to produce more uniform curd particle size than vertical double-O Damrow vats (Johnston, Luckman, Lilley, & Smale, 1998). The current authors have reported large variations in the moisture content of curd samples collected from pilot-scale cheese vats (Panthi et al., 2018). Everard et al. (2008) observed no influence of stirring speed on curd moisture levels during stirring in-vat but observed increased losses of casein fines and fat into whey with increasing stirring speed. Hence,

248 the principle of increasing cutting and stirring speed to reduce curd particle size and thus
249 influence syneresis properties may sometimes have negative consequences. This also supports
250 the case for consideration of alteration of knife geometry or size to achieve a desired final cheese
251 moisture content without incurring excessive curd losses. Everard et al. (2011) reported no
252 differences in final moisture content in cheese after pressing (22 hr), although curds moisture
253 contents prior to drainage were different, possibly due to use of long pressing times for a small
254 cheese mass.

255 Where multiple external factors exist, interactions between these factors are important.
256 Thomann, Brechenmacher, and Hinrichs (2006) observed an interaction between temperature
257 (25-60^{o}C) and sizes of the curds on curd syneresis during stirring and reported that increasing
258 temperature and decreasing particle size accelerated the syneresis. However, at temperatures
259 above 60^{o}C, the rate of syneresis can be slow because of denaturation of whey proteins (Calvo &
260 Balcones, 2000) or skin formation on the curd particles surface, closing the pores (Oberg,
261 Mcmanus, & Mcmahon, 1993). Moreover, decreasing milk pH (6.6 to 6.2) and increasing
262 cooking temperature (from 40 to 50^{o}C) accelerates the rate of syneresis (Giroux et al., 2014),
263 which also suggests that protein network rearrangement enhances curd contraction. However,
264 homogenizing milk prior to cheese-making retards syneresis (Thomann, Schenkel, & Hinrichs,
265 2008), possibly due to the adsorption of protein material on the fat globule surface, which
266 impedes network shrinkage. The highest level of shrinkage in curds can be observed with smaller
267 size (13, 16 or 21 mm) and lower pH (6.0, 6.2 6.4) of curd particles within the first 80 min of
268 syneresis as measured using volume of whey expelled from a single curd (Grundelius et al.,
269 2000). However, the effect of curd grain size on syneresis is less clearly observed when curds are
270 stirred for a long time (180 min) (Grundelius et al., 2000). Although it is highly unlikely that

curds slab be stirred for such a long time during cheesemaking, this indicates that the extent of curd contraction does not depend on its dimension, in contrast to what was reported by Lodiate et al. (2000). Thus, there is a need to have a greater understanding of the interactive effective effects of cheese-making conditions in improving process efficiency or in reducing process variation during cheese-making.

Milk from indoor or outdoor herd feeding systems showed minimal differences in moisture loss during in-vat stirring (Panthi et al., 2018). The resultant curds derived from milk of different herd feeding systems, standardized to similar levels of fat and protein, exhibit similar moisture loss kinetics (Figure 3). However, the rate and extent of moisture loss can be significantly different between curd samples derived from different feeding systems, when milk composition is not standardized.

6. Syneresis of curds prepared from milk with increased levels of solids

Protein concentration of milk can be achieved using ultrafiltration (UF) retentate or blending UF retentate into regular milk. Concentrating milk using membrane filtration technology offers opportunities to reduce the impact of variability in milk composition, and simultaneously increase processing capacity using the same resources (Thomann et al., 2008). However, concentration of milk results in significantly different physico-chemical properties in comparison to regular milk (Casiraghi, Peri, & Piazza, 1987; Ong et al., 2013a), which affects the curd firmness (Lucisano, Peri, & Donati, 1985) and syneretic properties (Peri, Lucisano, & Donati, 1985) during cheese-making, and thereby final cheese quality (Ong et al., 2013a).

Use of ultrafiltration for low-factor concentration (up to 2x) of milk for cheese-making has been reviewed recently (Kevany & Guinee, 2018). Such increased protein levels in milk

result in marked changes in the protein network structure; for example, gels prepared from ultrafiltration (UF) retentate (5.8% w/w protein) appear to have fewer pores and thicker protein strands compared to gels prepared from milk (Fig. 2 C, D) (Ong, Dagastine, Kentish, & Gras, 2010) or from less-concentrated retentate (3.7, 4 or 4.8 % w/w protein) (Ong et al., 2013a). Gels, curds or cheese prepared from UF-retentate contain larger aggregates of milk fat entrapped by a thick protein network compared to those gels prepared from regular milk (Ong et al., 2013a), owing to the aggregation of fat globules during UF. It could be possible that gels prepared from concentrated milk shrink less compared to those prepared from regular milk because of the formation of a less flexible casein network and a higher volume fraction of casein particles, retarding moisture expulsion. Therefore, when utilizing milk concentration, careful consideration is required to achieve both suitable gel structure and curd rigidity (Ong et al., 2010) (and thus cheese firmness) and appropriate levels of whey expulsion from the resultant curds (Rodriguez, Requena, Fontecha, Goudedranche, & Juarez, 1999).

However, the porosity of different milk curds prepared using UF-retentates (4%, 4.8% or 5.6% wt/w protein) appears to be similar after pressing (Ong et al., 2013a; Rovira et al., 2011), which could be attributed to fusion of casein micelles (Oberg et al., 1993) and the release of moisture during pressing. It is important to note that the porosity of the curd may also differ between the edge or at the centre of the curd particles, previous studies have not focused on this aspect. A large number of factors can thus influence gel microstructure properties, and an understanding of the interactive nature of these factors is necessary to achieve a suitable protein network to control whey expulsion behaviour during stirring in-vat.

Rennet-induced gels formed using concentrated milk attain higher firmness at a given time compared to gels prepared from regular milk (Lucisano et al., 1985; Ong et al., 2013a).

316 Lower gel strength results in higher losses of fat and protein into whey, due to the formation of
317 weak gels, whereas higher gel strength results in fracture of curd during cutting, causing
318 significant loss of casein fines into whey (Guinee, Pudja, & Mulholland, 1994). A rigid curd
319 entraps moisture strongly in the network, causing difficulty in moisture expulsion (Van Vliet,
320 Van Dijk, Zoon, & Walstra, 1991). Previous studies highlighted the importance of cutting gels at
321 uniform firmness during cheese-making where milk composition is variable (Mateo et al., 2009c;
322 Ong et al., 2013a). However, in industrial practice, rennet-induced gels are mainly cut after a
323 specific time, which creates challenges for processing of milk of variable or higher solids content
324 for cheese-making, although the expertise of a cheesemaker can be used to determine appropriate
325 gel firmness (Govindasamy-Lucey et al., 2011). Alternatively, use of instruments seems a more
326 promising approach to monitor gel firmness so as to accurately achieve a uniform coagulum
327 firmness (Mateo et al., 2009c; Thomann et al., 2008).

328 Studies have reported that rapid coagulation occurring when using concentrated milk can
329 be slowed, to some extent, by lowering coagulation temperature by 2-3^{o}C (Guinee et al., 1994),
330 homogenizing the cheese milk (Thomann et al., 2008) or sequestering the levels of insoluble and
331 free Ca present in milk (Casiraghi et al., 1987). Lowering coagulation temperature during
332 cheese-making was reported to have no significant influence on cheese composition, ripening
333 and sensory properties (Govindasamy-Lucey et al., 2011), whereas homogenization and Ca-
334 sequestration may alter biochemical properties of the final cheese.

335 Studies have shown that increasing the solids content in milk by microfiltration or
336 ultrafiltration result in slowed (Caron et al., 2001; Casiraghi et al., 1987; Thomann et al., 2008)
337 or unaffected (Peri et al., 1985) syneresis kinetics. The extent of whey expulsion from the
338 concentrated milk could be lower compared to regular milk. This could be either due to higher

339 total solids levels in milk or creation of a more compact network, reducing permeability.
340 However, Thomann, et al. (2008) showed that, when considering the amount of permeate
341 released during the filtration process, curd from concentrated milk actually released higher levels
342 of whey than normal milk. This indicates that ultrafiltration enhances overall syneresis during
343 cheese-making, as a result of permeate release before renneting, which could complement
344 syneresis processes in attaining desired curd moisture contents or drainage times. However, it is
345 not clearly understood if the endogenous syneresis pressure was responsible for the increased
346 amount of whey expulsion.

347 Dejmek and Walstra (2004) suggested that care should be taken when comparing levels
348 of syneresis between milk of varying compositions, due to differences in initial moisture content,
349 as different initial levels of moisture in curds may result in different rates of syneresis.
350 Monitoring relative change in moisture levels or whey expulsion or curd shrinkage levels in the
351 cheese curds has been examined in previous studies to facilitate comparisons (Grundelius et al.,
352 2000; Mateo et al., 2009c; Thomann et al., 2006). However, some other studies have directly
353 compared the results without considering the initial level of curd moisture (Calvo & Espinoza,
354 1999; Caron et al., 2001).

355 Concentration of cheese milk can reduce overall processing time, due to reduced
356 coagulation and syneresis times (Ong et al., 2013a). However, it is important to balance changes
357 in curd moisture, pH and other biochemical changes in order to achieve similar final cheese
358 quality, compared to cheese made from traditional processes.

359 **7. Moisture prediction models**

360 Accurate prediction of curd moisture level during cheese-making would be a key
361 development for controlling cheese-making process where there is a variable milk composition

(Jimenez-Marquez et al., 2005; Mateo et al., 2009b). Curd moisture levels have been used as a basis for validation of modern real-time syneresis-monitoring technologies (Mateo et al., 2009b). Measurement of moisture level of curd provides more information for curd drainage time, particularly in dealing with varying milk composition and process parameters.

Syneresis has previously been modelled recording the changes in one side of the curd e.g., change in height of a curd slab (Lodaite et al., 2000; Van Dijk, 1982, Van den Bijgraat, 1988) or porous media (Tijskens & De Baerdemaeker, 2004); however, modelling from a three-dimensional perspective is challenging due to changes in multiple factors influencing curd contractions and subsequent whey expulsion. For example, varying sized curd particles and conditions make it difficult to develop a theory-based model for curd syneresis (Jimenez-Marquez et al., 2005). Different empirical models that can better predict curd moisture are being continuously evaluated and compared (Giroux et al., 2014; Mateo et al., 2009b; Panthi et al., 2018; Thomann et al., 2008).

We previously demonstrated that data for moisture loss kinetics follow a power law model under static conditions of temperature and pH (Panthi et al., 2018); however, the same power law model did not show a good fit when the experimental conditions were varied in a different cheese vat (unpublished data). This suggests that empirical equations need to be customized to different cheese-making conditions. Thus there is a requirement for an empirical model which can explain the moisture loss data even when cheese-making conditions are varied. An optical sensor technology using broad spectrum waves has been reported to be suitable for prediction of syneresis kinetics (Castillo et al., 2006; Mateo et al., 2009b). It is suggested that this area requires a separate review, particularly focusing on modelling of syneresis kinetics.

Jimenez-Marquez, et al. (2005) studied modelling using a Neural Network (NN) method at industrial scale, in which 57 data points were recorded between milk production and cheese production to predict cheese moisture before drainage. In the NN, elimination of less influential factors was performed, and it was found that those factors which most strongly affect cheese moisture are curd particle size, pH, temperature and milk composition. As stirring and cooking conditions in industrial scale processes usually adhere strictly to a SOP, these processes are not expected to be variable. However, when changing the stirring conditions or cutting process, these factors can also significantly influence syneresis. For example, Mateo, et al. (2009a) reported stirring time, stirring speed, cutting speed, and fat levels, along with data generated from optical sensor gave good prediction of curd moisture in a model system.

A simple empirical formula with one or two parameters may be useful for predicting curd moisture. However, syneresis kinetics change during stirring and have been reported to fit first-order (Calvo & Balcones, 2000; Casiraghi et al., 1987; Grundelius et al., 2000; Kaytanli et al., 1994), second-order (Giroux et al., 2014), higher-order (Huber, Fertsch, Schreiber, & Hinrichs, 2001) models, or Darcy's law (Castillo et al., 2006; van den Bijgraat, 1988). In these studies, expulsion of whey was measured in small beakers (Calvo & Balcones, 2000; Caron et al., 2001; Piyasena & Chambers, 2003), in tubes (Giroux et al., 2014; Thomann et al., 2008) or as single particles from a one-dimensional perspective (Geng et al., 2011; Grundelius et al., 2000). Therefore, kinetics measured in larger scale experiments with changing experimental conditions will most probably differ to those studied at small scale reported in the literature.

8.0 Methodologies for syneresis measurement

A large number of studies relating to syneresis of curds have been carried out over the last four decades and aspects of this work have been reviewed (Dejmek & Walstra, 2004;

Walstra et al., 1985), which has recently been updated by Fagan, O'Callaghan, Mateo, and Dejmek (2017). However, details of methods of syneresis measurement have not been covered in the recent updates. Recent studies that have employed different methodologies are summarized in Table 1. Common methods include direct measurement of curd area or height, measurement of volume of whey exuded, or measurement of curd moisture content, while inline measurement of syneresis through light back-scatter is a relatively new approach.

8.1 Direct measurement of curd shrinkage (shape and size)

Laser systems (Lodaite et al., 2000), magnetic resonance imaging (Ozilgen & Kauten, 1994) and nuclear magnetic resonance (Tellier et al., 1993, Hansen et al., 2010) have been employed to quantify changes in curd shrinkage as a function of time, and hence syneresis. These non-destructive methods measure curd dynamics without physically disturbing the gel or curd, and, therefore, are suitable for measurement of endogenous syneresis. However, laser and MRI imaging are limited to measurement of only one- dimensional shrinkage of curd.

8.2 Image Analysis

In this technique, the change in the area of individual curd particles can be determined from high-resolution pictures. Digital micrographs are used for capturing images of single cheese curds during syneresis (Renault et al., 1997) or deformation (Geng et al., 2011). The camera is controlled from a computer, which facilitates mapping of the syneresis process for an extended period of time (Geng et al., 2011; Iezzi et al., 2012; Renault et al., 1997). However, small curd particles are difficult to distinguish using this imaging technology (Renault et al., 1997), particularly if the curd has a large proportion of fines.

8.3 Mesh sieve method

Curd particle size (and curd particle size distribution) can also be determined using mesh sieve of different sizes (12, 7.5, 5, 3 mm) stacked one above another, using a cascade approach. The highest mesh size is at the top and particles are allowed to follow downwards to the lower mesh sizes, with curd particles of different sizes being retained within different sieves (Johnston et al., 1991; Johnston et al., 1998). However, this technique has the disadvantage of clogging of mesh pores by curd particles and also may cause physical damage to soft curd particles.

8.4 Direct measurement of whey yield

Measurement of whey expulsion has been a widely-used technique to determine curd syneresis indices (Arango et al., 2015; Cipolat-Gotet, Cecchinato, Stocco, & Bittante, 2016). The cumulative volume of whey exuded as a function of time during syneresis can indicate syneresis properties. In such approaches, milk is coagulated in different beakers and curd is separated and whey yield is weighed over time (Marshall, 1982; Piyasena & Chambers, 2003). Direct whey measurement in a large volume is relatively challenging, because whey needs to be separated from the curds, which can induce pressure on the curd particles.

8.5 Proportion of whey in curd/whey mixture

More recently, samples of the curd/whey mix have been drawn through an inline sampler fitted to the cheese vat, and the proportion of whey that is immediately drained from the sample is used as a measure of syneresis at that time point (Costa et al., 2012; Everard et al., 2011; Fagan, Castillo, Payne, O'Donnell, Leedy, & O'Callaghan, 2007; Mateo, O'Callaghan, & O'Donnell, 2010; Mateo et al., 2009c; Talens et al., 2010). Sampling occurs during curd stirring, which ensures homogenous distribution of curds particles in the cheese vat. A typical trend of whey release is shown in Figure 4, which shows that the majority of whey release occurs within 15 min of cutting, and that the whey content in the sample of curd/whey mixture remains

relatively constant. However, it is hard to precisely measure the whey proportion in a sample from a cheese vat, since the curd particles tend to sediment in the cheese vat after releasing sufficient moisture.

8.6 Measurement of whey yield using tracer compounds

Zviedrans and Graham (1981) developed a tracer method to determine syneresis in curd. A high molecular weight blue dextran (2000 kDa) was added to whey/curd after cutting; the concentration of blue dextran in whey decreased during stirring, due to dilution by exuded whey. The concentration of blue dextran was measured by absorption at 620 nm over the syneresis time, and the amount of whey released could be predicted as:

$$V_{Whey,t} = V_{BW}\left(A_o/(A_t - 1)\right)$$ (Grundelius et al., 2000)

Where V_{whey}, is the total volume of whey expelled at time t, $v_{(BW),}$ is the initial volume of blue whey, A_o is the initial absorbance of blue whey, and A_t is the absorbance at time t.

The tracer method has been used in different studies (Grundelius et al., 2000; Piyasena et al., 2003) and has been validated for different fat levels (Talens et al., 2010). The disadvantage of using blue dextran is that it can attach to the surface of cheese curds, which may affect the accuracy of the result. A suggested alternative approach may entail addition of blue-dextran in cheese milk before coagulation, and measurement of the concentration of blue-dextran in curd samples. The increased concentration of blue-dextran (per unit cheese curd) over time could be used as an index of syneresis.

Castillo, Payne, Lopez, Ferrandini, and Laencina (2005b) studied the kinetics of fat globule dilution in whey during syneresis at industrial scale, as a means of estimating the kinetics of whey separation using an endogenous milk component as a tracer (milk fat). Alternatively,

474 endogenous milk components, e.g., riboflavin or tryptophan have been exploited as tracer
475 compounds (Fagan et al., 2011) and this approach shows potential for monitoring syneresis in
476 real-time in industrial cheese-making. However, validation of this method is necessary.

477 ***8.7 Measurement of whey using centrifugation***

478 Whey measurement has been carried out by centrifuging curds/gels at different stages.
479 Brown et al. (2012) centrifuged curd in two stages (500 x g for 15 min followed by 1000 x g for
480 15 min) and measured the amount of whey released over time. This technique may forcefully
481 expel whey through porous channels, and higher fat loss may also occur in the whey, leading to
482 the overestimation of syneresis. Furthermore, curd having different microstructures as induced
483 by different protein concentrations may be not suitable for measurement using these methods,
484 since whey may be physically entrapped within the dense protein networks.

485 ***8.8 Measurement of curd moisture***

486 Most studies have reported on whey expulsion kinetics, but more recent studies have
487 sought to quantify moisture loss (Table 1). Curd moisture content is an important parameter
488 which can be used to make decisions on appropriate curd drainage time. A curd sample is taken
489 from the cheese vat and immediately weighed into dried dishes, followed by moisture
490 evaporation in a hot air oven. This method is a reliable technique as long as the curd moisture
491 contents are measured immediately after sampling. An instrument with infra-red capability for
492 moisture content determination increases the speed of the method. However, using infra-red for
493 moisture content in duplicate samples is time-consuming and is not appropriate for a kinetics
494 study where many samples are collected in a short time.

495 ***8.9 Inline measurement of syneresis***

Use of process analytical technology in cheese-making can deliver relevant information about product quality parameters in real-time (Panikuttira, O'Shea, Tobin, Tiwari, & O'Donnell, 2018). In-line measurement of syneresis is a non-destructive technique which allows monitoring of syneresis dynamics in real-time in cheese-making vats (Fagan et al., 2007). The use of the CoAguLite^{TM} sensor (based on near infrared wave reflection) for monitoring of coagulation process to determine cut time has been well-established (Castillo, Gonzalez, Payne, Laencina, & Lopez, 2005a; O'Callaghan, O'Donnell, & Payne, 2000). However, its application is limited in monitoring the syneresis process due to its narrow field of view. The light waves generated through narrow-field view sensors were highly scattered after cutting the coagulum, which caused difficulty in receiving the signals from the reflected light (Fagan et al., 2007).

Large field view (LFV) light back-scattering optical sensors have shown promising results for monitoring coagulation and syneresis under different cheese-making conditions (Arango et al., 2015; Castillo et al., 2006; Fagan et al., 2007). The optical sensor is fitted to the cheese-vat and produces infrared light at 980 nm; the waves are passed through cheese curds in the cheese vat and the reflection of the light is detected in a spectrometer (Fagan et al., 2007). The schematic of the inline optical sensors assembly is presented in Figure 5. The detailed operation of this method has been described by Mateo, et al. (2009a).

The reflection ratio is calculated from the voltage of back-scatter light passed through milk divided by the initial voltage generated to transmit the light during stirring of curds (Mateo et al., 2009a). As the proportion of whey increases in the cheese vat, the reflection ratio of the light decreases. The relation of reflection ratio as a function of time can be predicted using the first-order equation:

$$R_t = R_\alpha + (R_o - R_\infty)e^{-k*t}$$

where Rt is the light backscatter ratio at time t, R α = is the light backscatter ratio at infinite time, Ro is the light backscatter ratio at time zero, and k is the kinetic rate constant (min^{-1}).

Prediction of moisture content using NIR technology has been reported to be highly correlated (R^2 = 0.94) with curd moisture content (Mateo et al., 2009a). Recently, NIR technology was employed to compare the syneresis kinetics between curds inoculated with varying levels of a exopolysaccharide-producing strain (Costa et al., 2012) and fat (Arango et al., 2015). Although this is a compelling technique in syneresis research, it requires advances in data handling and analysis techniques to overcome the limitations of the method. Moreover, this technique needs to be further investigated under a wide range of cheese-making conditions, (e.g., concentrated milk, change in temperature and pH) and its influence on equipment cleaning and sanitation at commercial level determined (Panikuttira et al., 2018).

Similarly, computer vision techniques and colour measurement have been investigated as a potential tool for the measurement of syneresis during in-vat stirring. The change in colour of curd during cheese-making has been found to be a good indicator of syneresis. Computer vision measures the red, green and blue colour components of curd and can distinguish between curds and whey and, in conjunction with image processing software, can give a measure of curd particle size and quantity, although it is limited to one dimension (Everard et al., 2007). Computer vision and NIR are thus potential technologies for monitoring syneresis processes (Mateo et al., 2010).

By analysing syneresis measurement methods, comparison of syneresis data from such methods could provide valuable information, and correlation of these methods with moisture content may be a useful approach to compare the accuracy of the methods.

9. Conditions used in syneresis experiments

Table 2 summarises the experimental conditions employed during syneresis or moisture loss studies. Studies have undertaken syneresis studies under different experimental conditions, e.g., stirring, shaking, and quiescent. All of these factors influence the moisture loss profile, since movement of curds causes faster whey expulsion compared to curds that are in quiescent conditions. Similarly, the quantity of milk for curd making to study syneresis during stirring also varies largely. For studying the detailed mechanism of curd contraction or endogenous pressure, use of a single curd particle can reduce the impact of other factors, such as collisions between curd particles and breakage. However, studies with larger milk volumes in a cheese vat may provide similar scenarios of cheese-making to the industrial practice. Similarly, the temperature in the studies has typically been maintained constant, and only a few studies have included the cooking process during stirring (Caron et al., 2001). Therefore, comparison of results between studies needs careful consideration, given the broad range of conditions and the methods used.

10. Conclusions

Syneresis, or whey expulsion, is one of the most important phenomena to consider during cheese-making, particularly when dealing with milks of varying compositions. There remains a requirement for greater understanding of endogenous syneresis and of casein micelle rearrangement in curds/gels which represent dynamic cheesemaking conditions. The interaction between variables employed determines whether syneresis is accentuated or slowed down, and

559 this can be exploited by the cheese producers to attain desired curd moisture contents by
560 changing the process parameters, particularly when concentrated milk is used.

561 Different methods have been employed to determine syneresis or moisture loss
562 properties. One of the easiest methods to measure syneresis is to determine the moisture content
563 of the curd. Determination of curd moisture content also facilitates decision-making for
564 appropriate curd drainage time or desired moisture content. The use of inline sensors has
565 especially gained interest in recent to achieve real-time prediction of curd moisture content
566 during stirring. Although there has been a remarkable development in prediction based on
567 empirical equations under fixed conditions, use of empirical equations under variable processing
568 conditions is still unproven. There is no doubt that accurate prediction of syneresis or moisture
569 provides a solution for eliminating uncertainties caused by change in milk composition, e.g.,
570 with seasonal milk production.

571 Various studies have used different experimental conditions, which precludes the
572 comparison of data between studies. Likewise, various methods for syneresis measurement exist,
573 and all of them have some limitations. This review advocates the determination of curd moisture
574 content as a method to provide accurate information about the rate of syneresis as well as the
575 suitability of curd for drainage during cheese manufacture. Furthermore; this method can also be
576 used as a benchmark to compare other methods and predictive models.

577 **Acknowledgment**

578 The authors thank Dr. Prateek Sharma for discussing valuable information required for
579 improving this manuscript. This work was supported by the Dairy Levy Trust Fund (grant
580 number 6259) administered by Dairy Research Ireland

581 Declarations of interest: none

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812 **Figure 1**. Schematic presentation of changes in storage modulus of milk recorded by a
813 rheometer after the addition of rennet (upper graph) and the corresponding changes in protein
814 network formation. Images were adapted from Horne and Lucy (2017) for hydrolysis and
815 aggregation and from Mellemma (2002) for the illustration of rearrangement.

816 **Figure 2.** Cryo SEM micrographs of milk gels coagulated at (A) 27 °C and (B) 36 °C using
817 regular milk and at different protein contents of (C) 3.7% and (D) 5.6%, at similar curd
818 firmness coagulated at 33^{o}C. Scale bars in A and B are at 20 µm and C and D at 10 µm. F and
819 P stand for fat and protein, respectively (adapted from Ong et al., 2011; Ong et al., 2013a).

820 **Figure 3**. Effect of feeding system on moisture loss properties of cheese curds during in-vat
821 stirring. C, G, T indicate milk derived from cows fed grass, grass/clover, and total mixed
822 ration, respectively (adapted from Panthi et al., 2018).

823 **Figure 4**. The proportion of whey in a curd/whey mixture during in-vat stirring as a function
824 of time (single line redrawn from Mateo et al., 2010).

825 **Figure 5**. Schematic presentation of an optical sensor used in measuring syneresis during
826 cheese-making (adapted from Fagan et al., 2007).

Table 1. Summary of approaches employed for measuring syneresis

Parameter	Method	Code*	References
Whey yield	Volume/weight of whey measured by filtering the entire content over time	1A	(Arango et al., 2015; Calvo & Balcones, 2000; Calvo & Espinoza, 1999; Caron et al., 2001; Cipolat-Gotet et al., 2016; Giroux et al., 2014; Huber et al., 2001; Kaytanli et al., 1994; Piyasena & Chambers, 2003; Thomann et al., 2008)
	Proportion of whey measured by an inline sampler as a function of time	1B	(Costa et al., 2012; Everard et al., 2011; Everard et al., 2008; Fagan et al., 2007; Mateo et al., 2009a; Mateo et al., 2010; Mateo et al., 2009c; Talens et al., 2010)
	Expulsion of whey volume measured using centrifugation	1C	(Brown et al., 2012; Daviau, Famelart, Pierre, Goudedranche, & Maubois, 2000)
	Volume of whey measured by tracer (Blue dextran) concentration as a function of time	1D	(Grundelius et al., 2000; Piyasena et al., 2003; Renault et al., 1997; Talens et al., 2010)
	Volume of whey predicted using riboflavin or tryptophan as a tracer in a prediction model	1E	(Fagan et al., 2011)
Curd shrinkage or contraction	Shrinkage of curd measured by change in area as a function of time using imaging technology	2A	(Geng et al., 2011; Iezzi et al., 2012; Renault et al., 1997)
	Shrinkage measured by laser displacement technique	2B	(Lodaite et al., 2000)
	One-dimensional shrinkage measured using magnetic resonance images	2c	(Ozilgen & Kauten, 1994)
Curd moisture	Determination of moisture content of fresh curd	3A	(Everard et al., 2011; Everard et al., 2008; Mateo et al., 2010; Mateo et al., 2009c)
Inline measurement	Computer vision, Light backscatter ratio (R)	4A	(Costa et al., 2012; Everard et al., 2011; Everard et al., 2008; Fagan et al., 2007; Mateo et al., 2009a; Mateo et al., 2010; Rovira, Garcia, & Lopez, 2012)

*Codes provided refer to the methods described in Table 2.

Table 2. Experimental conditions used in syneresis studies

Code	Milk types	milk /curd quantity used	Syneresis studied at	Stirring conditions	References
1A	Raw milk	50 ml	40°C	shake for 2 hrs	(Piyasena & Chambers, 2003)
	Milk	200 g	30-38-32°C	quiescent	(Caron et al., 2001)
	Reconstituted milk	NA	25-50°C	quiescent	(Kaytanli et al., 1994)
	Skim milk	4 pieces of curd	30°C	Shaken	(Huber et al., 2001)
	Skim milk	4-6 pieces of curd	30°C	Shaken	(Thomann et al., 2008)
	Raw milk	5 g	32°C	quiescent	(Giroux et al., 2014)
	Raw goat milk	4 pieces of curd	30°C	Shaken	(Thomann et al., 2006)
	Milk	100 ml	30°C	quiescent	(Calvo & Balcones, 2000)
	UF retentate	50 ml	38°C	quiescent	(Peri et al., 1985)
	Milk	50 ml	30°C	quiescent	(Calvo & Balcones, 2000)
1B	Recombined milk	11 l	32°C	Stirred for 75 min	(Mateo et al., 2009c)
1C	Skim milk	30 ml	33°C	quiescent	(Daviau et al., 2000)
1D	skim milk	Single curd	33°C	quiescent	(Grundelius et al., 2000)
	Skim milk	50 ml	40°C	Shaken	(Piyasena et al., 2003)
	Skim milk	250 ml	30°C	quiescent	(Renault et al., 1997)
2A	skim milk	Single curd	32°C	quiescent	(Geng et al., 2011)
	Hard cheese curds	Commercial curds		Stirred	(Iezzi et al., 2012)
2B	Skim milk	Single Curd slab	33°C	quiescent	(Lodaite et al., 2000)
2C	Whole milk	220 ml	30°C	quiescent	(Ozilgen & Kauten, 1994)
3A	Raw milk	100 ml	38°C	quiescent	(Casiraghi et al., 1987)
	Recombined milk	11 L	32°C	stirred for 75 min	(Everard et al., 2011; Everard et al., 2008)
4A	Milk	11 L	32°C	Stirred for 50 min	(Costa et al., 2012)
	Recombined milk	70 ml	27,32,37°C	quiescent	(Arango et al., 2015)

Refer to Table 1 for a description of methods employed.

NA refers to not available

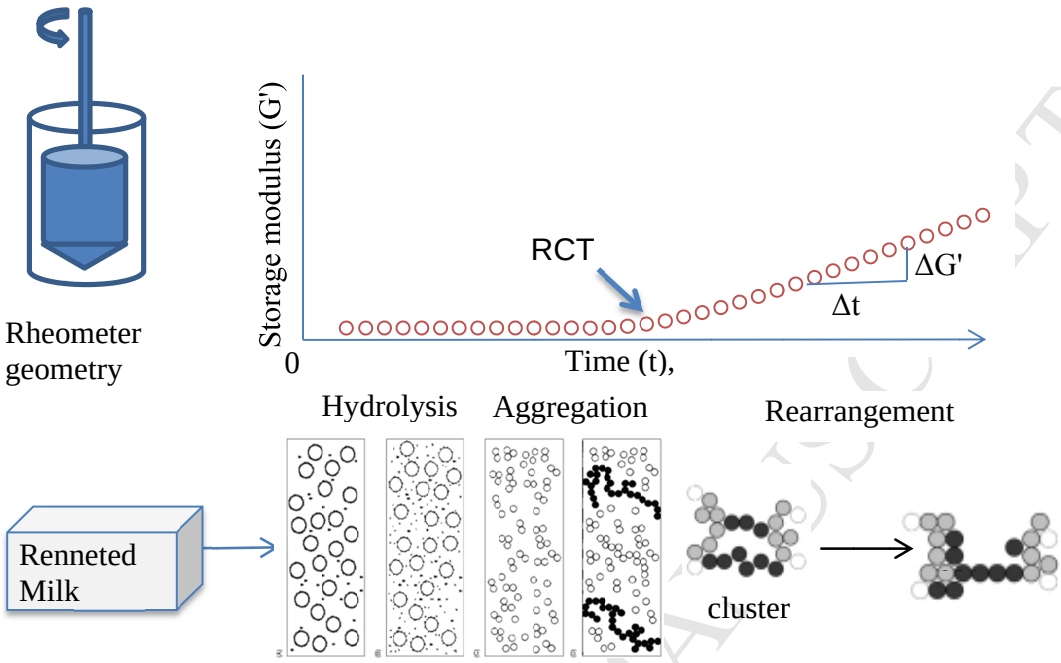

Figure 1

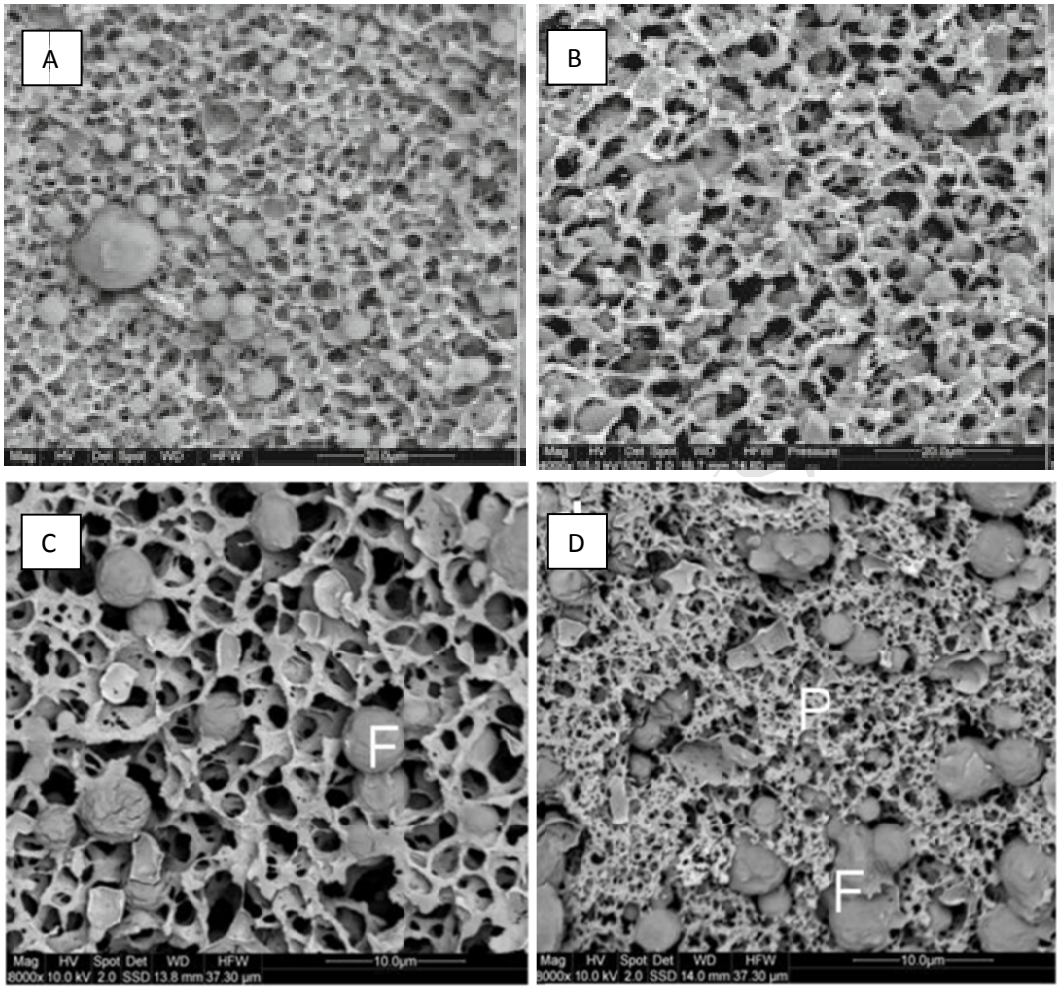
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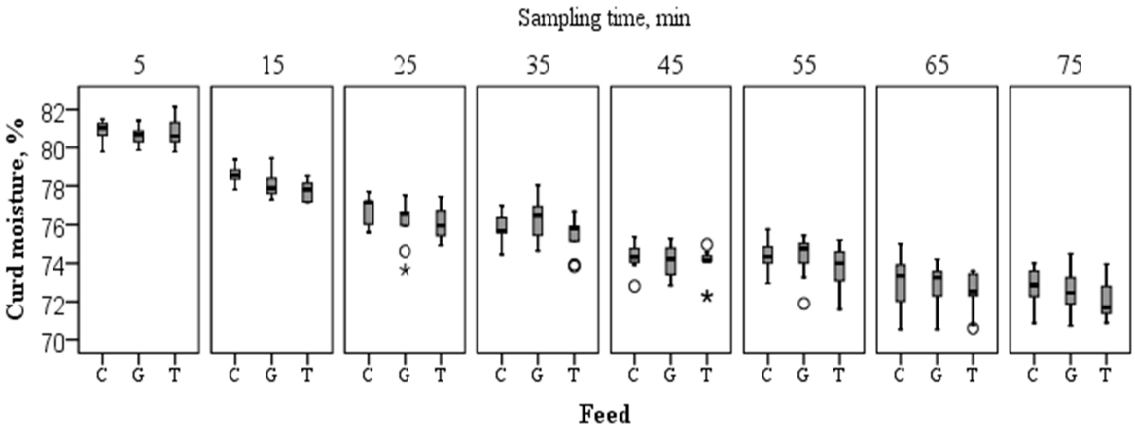
Figure 2.



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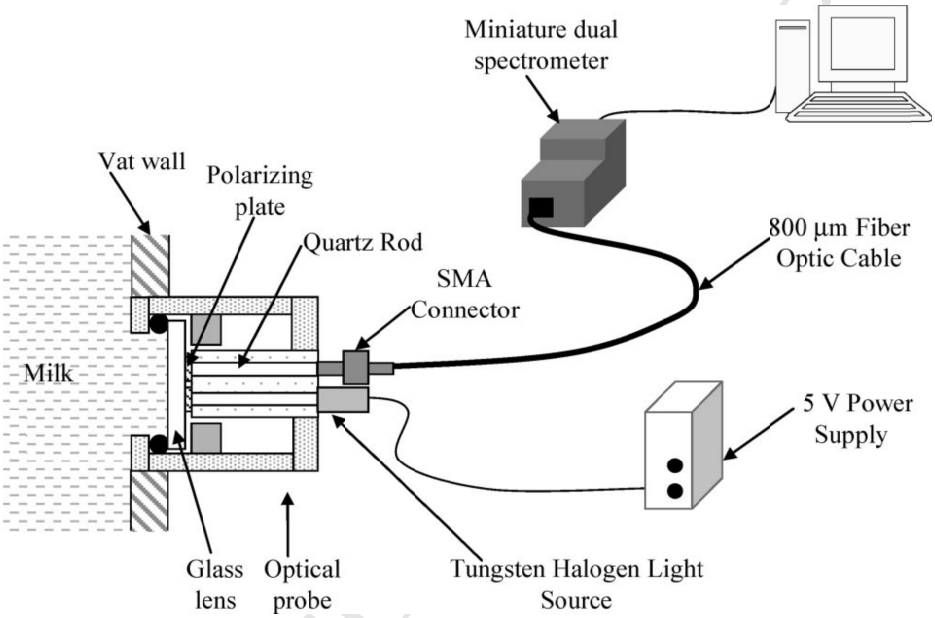

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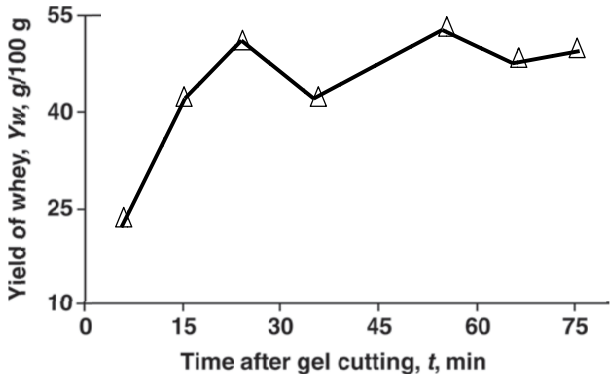

Figure 4.

Highlights

- Determining curd moisture content is a reliable technique for measurement of syneresis kinetics.
- Modelling for prediction of inline curd moisture content is of growing interest.
- The pattern of syneresis from protein-concentrated milk is complex.
- Endogenous syneresis under dynamic conditions requires greater understanding.
- Future studies should focus on interactive effects between variables.